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Urolithiasis and Transitional Cell Carcinoma of the Bladder in a Wistar Rat

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Urolithiasis and carcinoma of the urinary bladder are generally uncommon in rats. In particular, spontaneous urolithiasis and carcinoma of the bladder in young rats has not yet been reported. Our case concerns the unexpected finding of urolithiasis of the bladder and left kidney of an apparently healthy 4-mo-old male Wistar rat. The bladder was filled with 28 white, round to oval calculi of various sizes. The kidney presented a single unevenly shaped calculus in the pelvis. Histology revealed, in addition to urolithiasis, a grade II papillary transitional cell carcinoma of the bladder, with muscle invasion and neoplastic extension to the left renal pelvic epithelium. No previous experimental procedure or dietetic manipulation had occurred, which could be held responsible for these findings. These unusual findings indicate that, unknown to researchers, unapparent factors can affect the health status of even relatively young experimental animals. Asymptomatic disease, such as the unknown presence of a tumor, potentially can affect many parameters and thus influence research results.

The association between high animal protein consumption and the risk of urinary tract stone formation has long been recognized in humans.[6,29] Several risk factors for urinary stones have also been identified, such as increased urinary excretion of calcium, phosphorus, and magnesium.[2] Increased casein, as a source of excess protein, in the diet of Sprague-Dawley rats has been shown to result in hypercalciuria, which contributes to stone formation by increasing urinary saturation of calcium salts,[27] and in hypocitraturia, which can predispose animals to the formation of calcium-containing stones.[26]

Urolithiasis of the bladder is a well-documented risk factor for tumor development in humans and rodents and is considered the initiating event that leads to a hyperplastic response, followed by papillomas or diffuse papillomatosis, which may eventually become transitional cell carcinoma.[7,8,22] The incidence of tumors appears to differ among rat strains. A high prevalence of spontaneous urinary bladder tumors has been noted in Brown Norway rats.[5] Mature DA/Han rats (25 to 30 mo) have also been observed to develop spontaneous urinary bladder tumors, with a low incidence of stone occurrence in males.[11] Wistar rats rarely present spontaneous bladder tumors, and those that do are usually older than 2 y.[33] The unique occurrence of spontaneous urolithiasis and carcinoma of the bladder of a 4-mo-old Wistar rat is described in this case report.

Case Report

Our case concerns a 4-mo-old male Wistar rat (*Rattus norvegicus*) that was obtained at the age of 3 mo from the conventional breeding facility of the Demokritos National Center of Physics Research (Athens, Greece) and was conventionally housed in the animal house of the Laboratory of Experimental Surgery and Surgical Research (School of Medicine, University of Athens, Greece). The Laboratory is a licensed research facility, according to the Greek Presidential Decree 160/1991, with which the state conformed to the European Directive 86/609/EEC regarding "the protection of animals used for experimental and other scientific purposes." The rat was housed and cared for according to the Decree, with lights on from 0600 to 1800, room temperature of 20 to 22 °C, relative humidity of 50% to 70%, and 10 to 15 air changes per hour. Commercial pelletted diet (ELVIZ, Imathia, Greece) and tap water were available ad libitum.

The rat belonged to the control group of a research protocol (license no. K/328/31-1-03 from the Veterinary Directorate of Athens Prefecture) investigating the protective influence of protein C on experimental sepsis. This animal had received an intravenous administration of protein C without any surgical manipulation 1 wk after arrival. One month later, during which it was in good health, the rat underwent anesthesia with isoflurane and midline laparotomy for terminal exsanguination from the abdominal aorta, as were the other rats of the control group. Observation of the thickened urinary bladder wall during this procedure led to examination of the organ and the finding that it was filled with multiple (28) urinary stones. The stones varied in size and ranged from less than 1 to 8 mm in diameter. They had uneven porous surfaces, were round to oval in shape, were white, and had a total weight of 1.063 g (Figure 1). Grossly, the entire bladder wall was remarkably hyperplastic, with tiny papillary projections resembling a thick carpet.

Further examination of the urinary tract led to the discovery of an irregularly shaped stone in the pelvis of the left kidney. Both kidneys macroscopically had a normal external appearance; however, the right renal pelvis appeared hemorrhagic (Figure 2). The stones were sent for chemical analysis, and the bladder and kidneys were processed for histologic examination.

The rest of the animal's necropsy, as well as the total necropsies of the other 11 rats comprising the study's control group, were completed without any abnormal findings. All animals had been in good health prior to euthanasia, with regular food and water intake and increase in body weight. It is noteworthy that during the 26-y (1979 to present) activity of the Laboratory, where approximately 500 rats are used annually in noncarcinogenicity research studies, similar abnormal findings of either stones or hyperplasia of the urinary bladder have not been documented.

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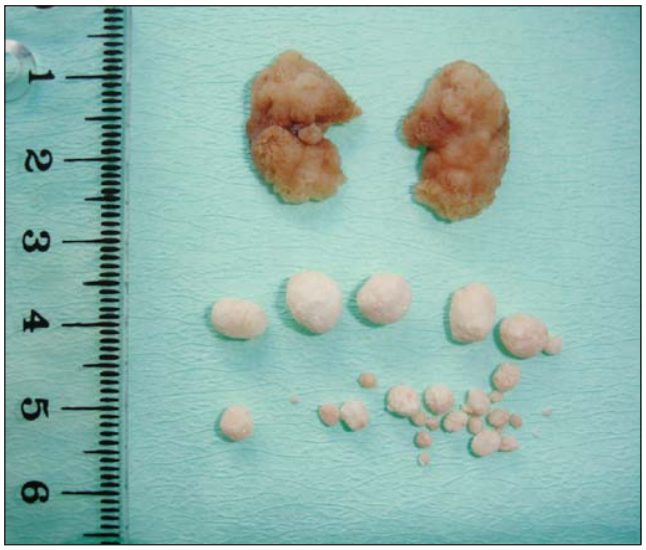

Figure 1. Gross appearance of the bladder, with pronounced thickness of the bladder wall and hyperplasia of the epithelium. The bladder and bladder stones are next to a centimeter ruler.

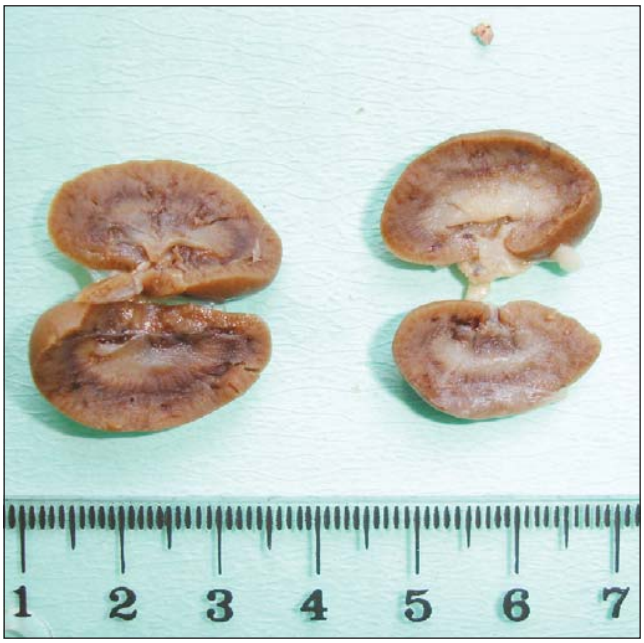

Figure 2. Gross appearance of the kidneys and kidney stone.

Results

Serum biochemistry analyses, such as alkaline phosphatase, urea, creatinine, calcium and phosphorus, carried out on the blood withdrawn from this rat were within normal limits.

Chemical analysis of the urinary stones confirmed that they were 20% calcium carbonate, 10% calcium oxalate, 30% phosphate salts, 10% urate salts (salts of uric acid), and 30% ammonium salts.

Microscopic examination of the bladder showed a hyperplastic, thickened bladder wall, which proved to be grade II transitional cell carcinoma. The surface of the tumor had a papillary pattern; the stroma was rich in newly formed vessels (angiogenesis); in addition, invasion of the lamina propia and the muscle wall were noticed (Figure 3). The cells were uniform in size, densely packed, and evenly distributed. The nuclei were slightly pleomorphic, with multiple nucleoli and frequent mitoses (Figure 4).

Microscopic examination of the kidneys revealed that the pelvic epithelium of the left kidney had a transitional cell carcinoma that extended from the bladder. The left renal medulla and cortex were free of tumor. Despite its hemorrhagic appearance, the right kidney lacked microscopic abnormalities.

Discussion

Urolithiasis has been observed to occur naturally and experimentally. Naturally occurring urolithiasis is an occasional finding mainly of old rats.[30,33] Several causes have been identified for the development of urinary calculi. Among these, certain diet ingredients have been shown to be responsible, such as increased casein,[2] choline,[10,17,18] calcium,[4] and vitamin A-deficient diets with lactose.[14] Certain animals, such as the genetic hypercalciuric stone-forming rats, develop urolithiasis even when fed a normal calcium diet.[13]

Experimentally induced urolithiasis has been developed by the administration of several agents, such as melamine,[20] terephthalic acid,[9] uracil,[23] manganese,[28] and biphenyl.[24] Experimentally induced urolithiasis has also been achieved by different surgical methods, such as bladder implantation of calcium oxalate stones[32] or zinc pellets[15] and catheter implants combined with spinal cord injury.[19]

The rat we present was not an aged animal and had not undergone any dietetic or surgical manipulation that could be held responsible for the urinary calculi. In our effort to resolve the etiology of the findings, we sought to investigate the diet this rat received at the breeding supplier until it was 3 mo of age, when our laboratory obtained it. The diet consisted of 16.5% protein, 2% fat, 8% fiber, 8% ash, 1% calcium, 0.6% phosphorus, and 1% sodium (Kounker, Athens, Greece). In comparison, the diet the rat received during its 1-mo stay at the university's animal house consisted of 21% protein, 6.2% fat, 4.5% fiber, 7.5% ash, 1.1% calcium, 0.9% phosphorus, 0.35% sodium, and 1.1% methionine; among the vitamins and minerals was 1000 mg/kg choline of unspecified source and production procedure, which was not included in the breeding diet.

We considered whether choline might be responsible for the appearance of the stones in our animal.[17,18] However, the rat received the choline-containing diet for only for 1 mo, which is less than the 8 wk that were necessary to produce symptoms after ingestion of high levels of choline, as reported by researchers in the United States.[18] It seems unlikely that the choline in the 2nd diet, at the concentration and duration given, could be the cause, since many rats in months-long research protocols in our laboratory have received this diet but have not presented similar abnormalities.

After examining the possible role of the diet provided, we then considered the role of urinary tract infection in producing calculi. Spontaneous infection of young Lewis rats by *Corynebacterium renale* has been reported to produce urinary calculi.[25] Experimental intracranial inoculation of lymphocytic choriomeningitis virus in Lewis rats has been shown to induce urolithiasis.[21] In addition, experimental inoculation of the kidney or bladder of Wistar rats with *Ureaplasma urealyticum* initiates stone formation, as early as within a few days.[3,35] This rapid initiation is especially noteworthy when one considers the size and number of stones found in our 4-mo-old rat. The possibility of spontaneous infection in the present case cannot be ruled out, as a culture sample was not processed and our rat had been bred and maintained in conventional facilities. However,

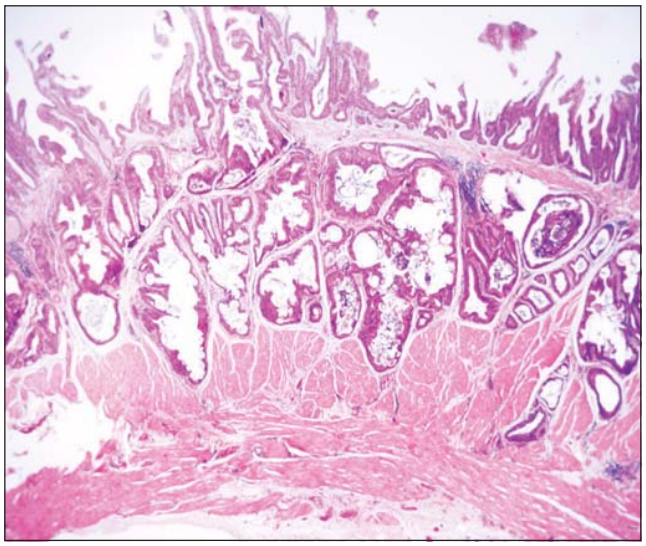

Figure 3. Microscopic image showing a papillary urothelial neoplasm on the surface of the bladder and invasion of the lamina propia and muscle wall. Hematoxylin and eosin stain; magnification, ×40 obj.

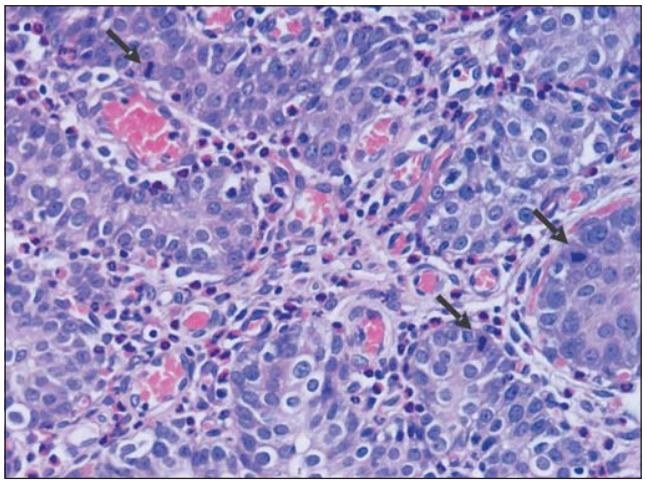

Figure 4. The neoplasm cells of the bladder are densely packed, evenly distributed, and have a uniform size. Their nuclei are slightly pleomorphic with nucleoli, and mitoses are numerous (arrows). Hematoxylin and eosin stain; magnification, ×40 obj.

necropsy of its cagemates showed they were free of disease.

We also investigated the possible etiology of our rat's bladder carcinoma. Urolithiasis of the bladder has been shown to be a risk factor for bladder epithelial proliferation and carcinogenesis.[7-9] There are some experimental studies of the model of portacaval shunted rats, where the correlation between urolithiasis and bladder lesions is conflicting.[1,16] Several chemicals, such as terephthalic acid, melamine, uracil, nitrosamine, nitrosourea, and arsenic, have carcinogenic potential for the rat urinary bladder.[9,20,22,23,34]

The rat we present had not been surgically manipulated or given any of the listed chemicals. However, it had received a single injection of protein C 1 mo before euthanasia. Administration of protein C to septic animals and humans has been shown to minimize disseminated intravascular coagulation, organ dysfunction, and death.[12] It is unlikely that its administration could be the cause of the pathologic findings. Given the fact that the rat had not received any carcinogenic chemical and that the single-dose administration of protein C is expected to have beneficial and not detrimental effects, we suspect that the bladder carcinoma developed secondarily to urolithiasis.

Regarding the renal carcinoma observed, tumors of the kidney are rare (1.5%) in the Wistar rat, even in carcinogenicity studies, and typically occur in animals older than 20 mo,[33] whereas this rat was only 4 mo old. Renal epithelial proliferation in other rat strains has been found to be as high as 59% in Brown Norway rats and 8% in Lewis rats. This proliferation concerned the medullary, as well as the papillary, wall of the renal pelvis.[31] The transitional cell carcinoma of the renal pelvis of this Wistar rat extended from the bladder.

We assessed this case in view of the current literature regarding spontaneously occurring stone production and carcinogenesis of the urinary tract as well as calculi resulting from environmental effects and experimental procedures. We have been unable to resolve the etiology of our findings. The most probable explanation for this case is an asymptomatic urinary tract infection of this conventionally bred and maintained rat, which may have led to stone formation and subsequent carcinogenesis. The rapid development and extent of these findings in a rat so young is noteworthy and, according to available literature, unique. It is unfortunate that culture samples could not be obtained after the discovery of the abnormal findings, as nonaseptic procedures for terminal exsanguination and necropsy had been followed. It is obvious that for microbiological documentation of this hypothesis in the future, adhesion to aseptic conditions during all terminal procedures is optimal. This practice depends, of course, on an institution's available facilities and funds.

We conclude that unapparent and thus unknown factors can affect the health status of even relatively young experimental animals. An asymptomatic disease, such as the presence of tumor, potentially can affect many parameters and influence research results. We believe that both researchers and laboratory animal scientists should maintain increased vigilance for unexpected findings and, if possible, routinely conduct comprehensive aseptic autopsies of their animals.

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